

Attorney Docket No.: 0800-0026  
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PATENT

Attached hereto is a marked-up version of the changes made to the specification by the current amendment. The attached page is captioned "**Version with markings to show changes made.**"

#### REMARKS

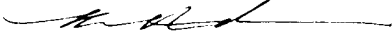
The foregoing amendments are made to insert the sequence identification numbers into the specification. In particular, the known VEGF<sub>165</sub> sequence is provided at amino acid positions 27-191 of SEQ ID NOS:1 and 2. See, e.g., Ferrara et al., *Endocrine Reviews* (1992) 13:18-32, provided with the Information Disclosure Statement submitted when the application was filed. Moreover, the sequences for the primers found at page 25 of the application have been added in the accompanying sequence listing and sequence identification numbers inserted into the specification.

Entry of the foregoing amendments is respectfully requested.

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Version with markings to show changes made

In the specification:

The paragraph beginning on page 3, line 14 has been replaced with the following rewritten paragraph:

--One such angiogenic factor, which specifically binds to and activates vascular endothelial cells, is vascular endothelial growth factor (VEGF). VEGF is a potent vasoactive protein. Four different molecular variants of VEGF have been described. The 165 amino acid variant (amino acids 27-191 of SEQ ID NOS:1 and 2) is the predominant molecular form found in normal cells and tissues. A less abundant, shorter form with a deletion of 44 amino acids between positions 116 and 159 (VEGF<sub>121</sub>), a longer form with an insertion of 24 basic residues in position 116 (VEGF<sub>189</sub>), and another longer form with an insertion of 41 amino acids (VEGF<sub>206</sub>), which includes the 24 amino acid insertion found in VEGF<sub>189</sub>, are also known. VEGF<sub>121</sub> and VEGF<sub>165</sub> are soluble proteins. VEGF<sub>189</sub> and VEGF<sub>206</sub> appear to be mostly cell-associated. All of the versions of VEGF are biologically active. --

The paragraph beginning on page 24, line 18 has been replaced with the following rewritten paragraph:

--Male Sprague-Dawley rats were anesthetized with an intraperitoneal injection of sodium pentobarbital (50 mg/kg). A longitudinal incision was made in the right thigh, after which the right femoral artery was surgically excised to induce limb ischemia. Rats were then transduced with rAAV-hVEGF<sub>165</sub> ( $2 \times 10^{13}$  virions; n=8) via intramuscular injection at sites within the ischemic hindlimb and also at sites within the contralateral limb. The vector suspension (100  $\mu$ L/site) was injected into 4 different sites in the major thigh muscles (quadriceps and adductor). Three rats received sham operations for preliminary assessment of hemodynamic examination. To confirm VEGF expression and to assess the possibility that VEGF was expressed in remote tissues, reverse transcription-polymerase chain reaction (RT

PCR) was performed on total RNA isolated from the ischemic hindlimb. Total RNA was isolated using RNA STAT-60 (TET-TEST, Inc., Friendswood, Tex.). Extracted RNA was

treated with DNase I (Takara Shuzo Co., Tokyo, Japan) to eliminate DNA contamination. The synthesis of first-strand cDNA was performed under conditions recommended in the ProSTAR First Strand RT-PCR kit (Stratagene, La Jolla, Calif.). The PCR amplifications were performed using human VEGF-specific primers (sense: 5'-GAGGGCAGAATCATCACGAAGT-3' (SEQ ID NO:3); antisense: 5'-CCACCTTCTTGATGTCATCA-3') (SEQ ID NO:4). GAPDH mRNA served as an internal standard. The PCR products were electrophoresed on ethidium bromide-stained 2.0% agarose gels. VEGF gene expression was observed 4 and 10 weeks after injection (FIG. 4 shows the results). On the other hand, no VEGF gene expression was detected in AAV-LacZ-transduced muscle. VEGF gene expression was not detected in the brain, heart, liver, spleen, kidney, and testes in rAAV-hVEGF<sub>165</sub>-treated rats 4 weeks after injection (FIG. 4).--